Molecular imaging of estrogen receptors

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Chapter 3

^{18}F-FES-PET as diagnostic tool in patients with breast cancer

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ABSTRACT

16α-[^{18}F]fluoro-17β-estradiol (^{18}F-FES) is an estrogen receptor (ER) specific positron emission tomography (PET) tracer with various potential interesting applications. The precise contribution of this technique in current clinical practice, however, has yet to be determined. Therefore the aim of this study was to evaluate the value of ^{18}F-FES-PET in breast cancer patients presenting with a clinical dilemma.

^{18}F-FES-PET examination could be requested by referring physicians for patients with a history of ER positive breast cancer, and presence of a clinical dilemma despite complete standard work-up. All requests for ^{18}F-FES-PET required a positive arbitration by a dedicated medical oncologist and nuclear medicine physician. The referring physician was asked to fill in validated questionnaires before, shortly after, and > 3 months after ^{18}F-FES-PET to determine indication, diagnostic value and therapeutic consequences of ^{18}F-FES-PET. To further validate ^{18}F-FES-PET findings, ^{18}F-FES-PET lesions were quantified and compared to centrally-reviewed conventional imaging.

Thirty-three patients underwent ^{18}F-FES-PET between December 2008 and October 2010. ^{18}F-FES-PET was requested to evaluate 1) equivocal lesions on conventional work-up (*n*=21), 2) ER status in metastatic patients (*n*=10), and 3) the origin of metastases (*n*=2). ^{18}F-FES-positive lesions were observed in 22 patients. ^{18}F-FES-PET was especially sensitive for bone metastases detecting 341 bone lesions compared to 246 by conventional imaging. The sensitivity for liver metastases was poor. In only 2 out of 7 patients with known liver metastases, these were detected on ^{18}F-FES-PET. Quantification of ^{18}F-FES uptake in liver lesions was hampered by high physiological background. ^{18}F-FES uptake was highly variable between all metastases (range SUV_{max} 1.7–18.8) and 45% of the patients with a positive ^{18}F-FES-PET had both ^{18}F-FES positive as well as ^{18}F-FES negative metastases. ^{18}F-FES-PET contributed to an improved diagnostic understanding in 88% of the patients and contributed to a change in therapy in 48% of the patients.

With the exception of liver metastases, whole-body imaging of ER expression with ^{18}F-FES-PET can be a valuable additional diagnostic tool when standard work-up is inconclusive. ^{18}F-FES-PET supported therapy decisions by improving diagnostic understanding and providing information on ER status of tumor lesions.

INTRODUCTION

Breast cancer is the most common cancer in women in the Western world.[97] Approximately 75% of the tumors express the estrogen receptor α (ER) at diagnosis.[98] Knowledge of the ER status of a patient has important consequences for treatment decision-making, as patients with ER positive tumors are likely to respond to antihormonal therapy.[21]

Evaluation of ER status is therefore performed by means of immunohistochemical staining of the primary tumor. This golden standard has some limitations: It predicts tumor response to antihormonal therapy correctly in only 50-60% of the patients,[99,100] the technique is semi-quantitative which can result in inter-observer variation, and ER scoring depends on the antibody used and delay-to-fixation time.[101,102] The recent systematic review by the American Society of Clinical Oncology and the College of American Pathologists revealed that up to 20% of all immunohistochemical determinations worldwide are inaccurate.[103] Absence of ER expression does, however, have a strong negative predictive value for response to antihormonal therapy.[22]

Also during metastatic disease, evaluation of ER status is important to determine changes in receptor expression. This is of relevance as discordant ER expression between primary tumor and metastatic lesions occurs in 18-40% of the patients.[8,9] A recent study in 336 patients with an ER positive primary tumor revealed loss of ER expression in distant metastases in 36% of these patients, which was a predictor of poor response to antihormonal therapy.[104] Therefore ESMO and NCCN guidelines recommend repeated biopsies.[105,106] In addition, biopsies of suspected recurrences are advised to confirm the diagnosis of metastasized breast cancer and to exclude benign lesions or (metastases from) a second cancer.

Despite these clear indications for rebiopsy, this may not always be feasible due to the characteristics of the lesion (such as location) or the patient (such as co-morbidity). In addition, within a single tumor or across lesions within a patient, ER expression can be heterogeneous.[15] In these cases a single biopsy may not be representative for the ER characteristics of the tumor burden as a whole. Furthermore, physicians may be reluctant to perform biopsies given the invasive nature of the procedure. A noninvasive method to quantify ER expression in all metastatic lesions would therefore be valuable.

Whole-body Positron Emission Tomography (PET) with 16α-[^{18}F]fluoro-17β-estradiol (^{18}F-FES) provides a unique method to non-invasively obtain molecular information about ER expression.[71,107] Several studies have shown that ^{18}F-FES-PET can reliably detect ER positive tumor lesions and that ^{18}F-FES uptake correlates well with immunohistochemical scoring for ER.[10,16,96] Furthermore, low ^{18}F-FES uptake was a strong predictor for failure of antihormonal

therapy.[63,64,67] Based on these results ^{18}F-FES-PET examination could since December 2008 routinely be requested in our center for patients with a history of ER positive breast cancer in whom a clinical dilemma remained present despite complete standard work-up. For example, when imaging procedures were inconclusive and performing a biopsy was not feasible. The aim of this study was to determine the value of ^{18}F-FES-PET in breast cancer patients presenting with a clinical dilemma.

METHODS

Patient selection

Patients with a history of histologically proven ER positive breast cancer were eligible for routine ^{18}F-FES-PET examination when, despite complete standard work-up, they presented a clinical dilemma for their treating physician. All requests for ^{18}F-FES-PET required a positive arbitration by a dedicated medical oncologist and nuclear medicine physician. From all patients a detailed medical history, current complaints, laboratory results (if available), and conventional imaging results were collected. The informed consent requirements for these retrospectively enrolled patients were waived by the UMCG Institutional Review Board (METc 2010.102).

Standard work-up

The routine staging protocol for these patients adheres to the Dutch Breast Cancer Guidelines, which are highly comparable with NCCN guidelines v1.2012.[105] In short, work-up for disseminated disease includes chest-CT or chest imaging, CT-abdomen or ultrasound of the liver and bone scan. During follow-up, examinations are directed by signs and symptoms and include bone scan or MRI in case of localized bone pain or elevated AF; chest-CT in case of chest lesions or pulmonary complaints, and CT-abdomen in case of abdominal lesions or abnormal liver tests. The use of ^{18}F-FDG-PET is discouraged and limited to patients with equivocal lesions, although tissue biopsy is more likely to provide useful information in these cases. Biopsies of suspected distant recurrences are recommended to confirm the diagnosis of metastatic breast cancer, and evaluate receptor status.

^{18}F-FES-PET imaging

ER antagonists were discontinued for a minimum of 5 weeks prior to ^{18}F-FES-PET imaging to prevent false negative results. The use of aromatase inhibitors was allowed. ^{18}F-FES was produced as previously described.[108] ^{18}F-FES ready for injection was obtained in 32 ± 10% decay-corrected radiochemical yield. Specific activity was 182 ± 101 MBq/nmol with a radiochemical purity 99.9 ± 0.3%. Patients received 207 ± 8 MBq ^{18}F-FES intravenously.

Whole body ^{18}F-FES-PET was performed 60 min after tracer injection, using a Siemens Ecat Exact HR+ PET camera (Siemens CTI, Knoxville, TN) with 5 mm spatial resolution with an emission time of 5 min and a transmission time of 2 min per bed position, or a Siemens 64 slice mCT (PET/CT) camera (Siemens CTI) with 2 mm spatial resolution with an emission time of 3 min per bed position and a transmission CT for attenuation correction. All scans and quantifications were performed according to the EANM guidelines for tumor PET imaging.[27] Scans were reconstructed with a 5-mm FWHM Gaussian reconstruction filter and iterative reconstruction methods were used with 3 iterations and 24 subsets. PET images were assessed qualitatively and quantitatively by a nuclear medicine physician. In reference to other ^{18}F-FES-PET studies, we used the maximum standardized uptake value (SUV_{max}) to quantify ER expression and a cut-off value of ≥1.5 to dichotomize results in ER positive and ER negative.[10] When in the field of view, CT data was used to allocate PET-positive lesions to an anatomical substrate.

Analysis of imaging results

Imaging analysis was performed retrospectively. Lesions detected by ^{18}F-FES-PET were recorded and ^{18}F-FES uptake was quantified. When a patient had innumerable lesions, an arbitrary maximum of 40 lesions were recorded. Aside from evaluation of the routine radiology and nuclear medicine reports, conventional imaging was centrally re-evaluated, while investigators were blinded for other imaging results. All lesions were classified into benign, equivocal and metastatic lesions. Thereafter, conventional imaging results were compared to findings on ^{18}F-FES-PET. For discordant lesions, fusion of ^{18}F-FES-PET with CT, MRI or ^{18}F-FDG-PET, and one-to-one comparison between ^{18}F-FES-PET and bone scan, was performed for final classification of these lesions. Follow-up imaging and clinical data were reviewed, whenever available, to evaluate remaining discordances.

Clinical value

Validated questionnaires were used to collect the insight of the referring physician before, shortly after, and > 3 months after ^{18}F-FES-PET imaging (supplemental table).[109] Questionnaire 1 served to identify the clinical dilemma and intended therapeutic strategy. Based on the reason for ^{18}F-FES-PET examination, the patients were retrospectively categorized into three different groups. Questionnaire 2 permitted to evaluate the outcome of ^{18}F-FES-PET, and the influence on treatment decision-making. The last questionnaire served to analyze the value of ^{18}F-FES-PET on diagnostic understanding and therapy-management after a follow-up of at least 3 months. The scoring was performed by the referring physicians. All questionnaires were checked for internal consistency.

Statistical analysis

Differences in tracer uptake between different (patients and) organs were analyzed by a one-way ANOVA. Site-to-site variability in ^{18}F-FES uptake was expressed as coefficient of variability. Scores on diagnostic understanding and therapeutic consequences were calculated for the three different reasons for ^{18}F-FES-PET examination with a two-sided non-parametric Kruskal-Wallis test.

RESULTS

Patient characteristics

Thirty-three patients were referred for ^{18}F-FES-PET examination between December 2008 and October 2010. Patients were retrospectively divided into three groups based on the reason for ^{18}F-FES-PET examination: 1) to differentiate between benign and malignant lesions in case of equivocal or ambiguous work-up (*n*=21), 2) to evaluate the patients ER status (*n*=10), and 3) to differentiate between metastases originating from different tumor types (n=2). Patient characteristics are shown in table 1.

Table 1: Patient characteristics

Characteristic	Category I (n=21)	Category II (n=10)	Category III (n=2)	All patients (n=33)
Age, years				
Mean	57	61	55	58
Range	43-78	48-77	54-56	43-78
Menopausal status				
Premenopausal	1	0	0	1
Postmenopausal	16	10	2	28
Unknown	4	0	0	4
Breast Cancer Stage				
Suspected distant recurrence	12	0	2	14
Metastatic disease	9	10	0	19
Prior lines of endocrine therapy*				
0	13	0	2	15
1	5	4	0	9
>1	3	6	0	9
Years between primary diagnosis and ^{18}F-FES-PET				
Mean	8	10	3	8
Range	0-18	3-22	2-4	0-22

*^{18}F-FES-PET examination was requested to evaluate equivocal or conflicting conventional imaging (Category I), a patients ER status (Category II), or the origin of metastases (Category III); *adjuvant therapies excluded*

^{18}F-FES-PET and conventional imaging results

^{18}F-FES-PET was performed in 22 patients and ^{18}F-FES-PET/CT in 11. Work-up prior to ^{18}F-FES-PET consisted of CT (n=24), bone scan (n=23 patients), MRI (n=8) and ^{18}F-FDG-PET

(n=3). Ultrasound and X-ray images were not taken into account in our analysis. Biopsy of suspicious lesions was performed prior to ^{18}F-FES-PET in four patients and during follow-up in two. ^{18}F-FES-PET showed ^{18}F-FES-positive metastases in 22 of the 33 patients. No apparent false positives were noted in a patient-based analysis.

^{18}F-FES-PET was negative in 11 patients. In three patients ^{18}F-FES-PET showed no tumor lesions despite the presence of metastases. This matched the histological findings (ER negative) in tumor biopsy in two patients. In one patient, 3 months later biopsy showed an immunohistochemical ER positive metastasis in the liver. In the remaining eight patients the negative ^{18}F-FES-PET concurred in the absence of metastases during follow-up of >6 months.

A total of 398 lesions were detected by ^{18}F-FES-PET. Lesions were located in bone (n=341 lesions), lymph nodes (n=26), lung and/or pleura (n=19), liver (n=8) and soft tissue (n=4). While being blinded for ^{18}F-FES-PET, investigators detected 242 of these 398 ^{18}F-FES positive lesions (61%) on conventional imaging. After comparison between conventional imaging and ^{18}F-FES-PET, an additional 79 lesions with ^{18}F-FES uptake could also be detected on conventional techniques. Of the remaining lesions, 15 were confirmed during follow-up. Therefore, the majority of all ^{18}F-FES-PET lesions (n=336, 84%) could eventually be validated by conventional techniques. The remaining 62 lesions were not detected during follow-up. However, nearly all these lesions were additional bone metastases in patients with known bone metastases. It is therefore plausible that these lesions are also true-positives.

Investigators detected a total of 319 metastases on conventional imaging, while being blinded for ^{18}F-FES-PET. Out of these, ^{18}F-FES-PET missed 77 lesions (24%). Detection of liver metastases by ^{18}F-FES-PET was poor. None of seven patients with known liver metastases had increased focal ^{18}F-FES uptake. In two patients, however, cold spots were observed at the site of liver metastases, and in two others ^{18}F-FES uptake was more heterogeneous than in other patients. Also relatively few lymph nodes were detected by ^{18}F-FES-PET (26 of 55, 47%). However, 68% of the undetected nodes had diameters < 15 mm (RECIST v1.1 cut-off value for measurable lymph node metastases), indicating that some of these may have been misclassified, or were below the detection threshold for PET imaging.[110] An overview of the concordance between conventional imaging and ^{18}F-FES-PET is shown in table 2.

Heterogeneity of ^{18}F-FES uptake

A striking 11-fold difference in tracer uptake was observed between different patients (range SUV_{max} 1.7–18.8), and a 6-fold difference between lesions within the same individual (range SUV_{max} 2.6–15.8). The coefficient of variability for the SUV_{max} of all ^{18}F-FES-PET lesions was high (68% ± 4%, 95% confidence interval). In 45% of the patients, both ^{18}F-FES positive and ^{18}F-FES negative metastases were present, suggesting partial discordant ER expression. Only

Table 2: Malignant lesions detected at conventional imaging and ^{18}F-FES-PET

Location	Conventional imaging*	^{18}F-FES-PET concordant with conventional imaging	Total ^{18}F-FES-PET	Conventional imaging concordant with ^{18}F-FES-PET	
				With masking of ^{18}F-FES result	With knowledge of ^{18}F-FES result**
Bone	246	212 (86)	341	212 (63)	292 (86)
Lung	9	7 (78)	19	7 (37)	13 (68)
Liver	20	8 (40)	8	8 (100)	8 (100)
Lymph nodes					
< 15mm	27	7 (26)	7	7 (100)	7 (100)
> 15mm	16	7 (44)	19	7 (37)	12 (63)
Soft tissue	1	1 (100)	4	1 (75)	3 (75)
Total	**319**	**242 (76)**	**398**	**242 (61)**	**336 (84)**

**Lesions considered malignant after central revision, while reviewers were masked for ^{18}F-FES-PET result.*
***Lesions considered malignant after comparison with ^{18}F-FES-PET was allowed, and including 15 lesions detected at follow-up shortly after ^{18}F-FES-PET (<1 mo)*. Data in parentheses are percentages.

one patient, had conversion to a complete ER negative phenotype, indicated by absence of ^{18}F-FES uptake in all (n=4) tumor lesions. Quantification of ^{18}F-FES uptake in liver metastases was hampered due to high physiological uptake in surrounding normal liver tissue. Mean ^{18}F-FES uptake did not differ significantly among metastases in different organs (figure 1). Only one premenopausal patient underwent ^{18}F-FES-PET in this study. She had tracer uptake (SUV range 1.2- 1.6) in tumor lesions far below the 95% confidence interval (5.9 ± 0.4) of the postmenopausal patients. Although it may be theoretically plausible that ^{18}F-FES uptake depends on estrogen background level, this cannot be concluded from these limited data.

Clinical Value of ^{18}F-FES-PET

^{18}F-FES-PET in case of equivocal or conflicting standard work-up

Twenty-one patients underwent ^{18}F-FES-PET to evaluate equivocal or ambiguous findings on standard work-up, or for signs and symptoms for which no substrate could be detected on conventional imaging. Suspicious lesions were present in bone (n=13), lung (n=4), liver (*n*= 3), and abdomen (n=1). These patients underwent bone scan (n=16), CT-scan (n=16), MRI (n=4) ^{18}F-FDG-PET (n=3) and biopsy (n=3) prior to ^{18}F-FES-PET, which did not establish a final diagnosis. In nine of these, ^{18}F-FES-PET showed elevated uptake in the suspected lesions, confirming the presence of ER positive metastases. Interestingly, in three patients biopsies prior to ^{18}F-FES-PET did not show malignancy despite suspected distant recurrences on conventional imaging. ^{18}F-FES-PET showed multiple ^{18}F-FES-positive lesions in these three patients (see figure 2 for an example). The next therapeutic choice was affected by ^{18}F-FES-PET in seven out of nine patients with positive ^{18}F-FES-PET findings. Four of them received radiotherapy, and one received bisphosphonates, in addition to the intended antihormonal

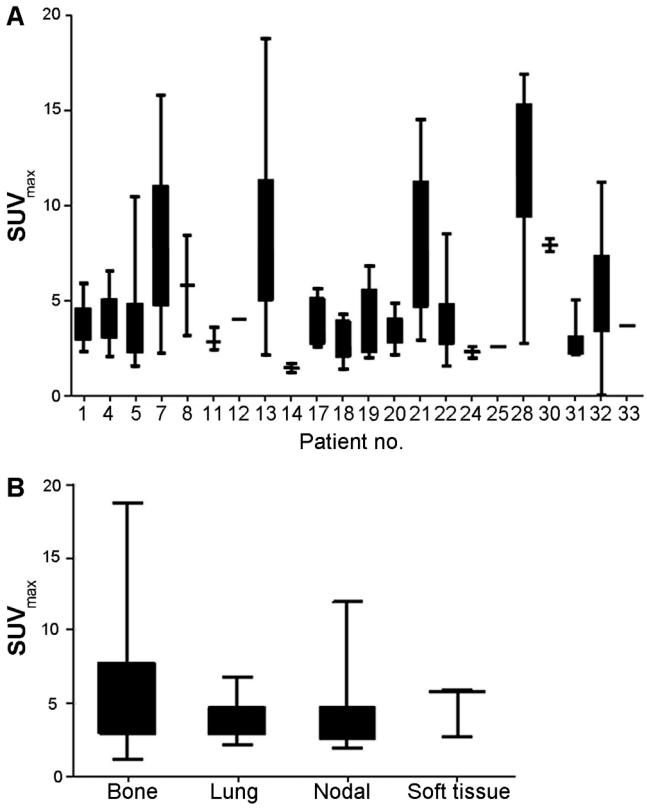

Figure 1. Differences in tracer uptake in all 22 patients with positive lesions on ^{18}F-FES-PET (A) and tracer uptake at different sites of metastases (B).

therapy, after ^{18}F-FES-PET provided confirmation of bone metastases. In two patients first-line antihormonal therapy for metastatic disease was initiated. In the remaining 12 patients, ^{18}F-FES uptake was absent at the suspected sites. Follow-up of at least 6 months of the suspected lesions, indicated no metastasis in 10 of 12 patients. In two patient conventional imaging remained suspicious and biopsy was performed. Histology showed ER negative adenocarcinoma in one patient and an ER positive liver metastasis in the other patient. In two patients ^{18}F-FES-PET was negative at the suspected site, but revealed unknown metastases at other sites. ^{18}F-FES-PET affected therapy management in only two patients with ^{18}F-FES-negative findings. In these two, ^{18}F-FES-PET led to refraining from radiotherapy in the absence of ^{18}F-FES uptake at the suspected lesions and confirmation of benign disease during follow-up.

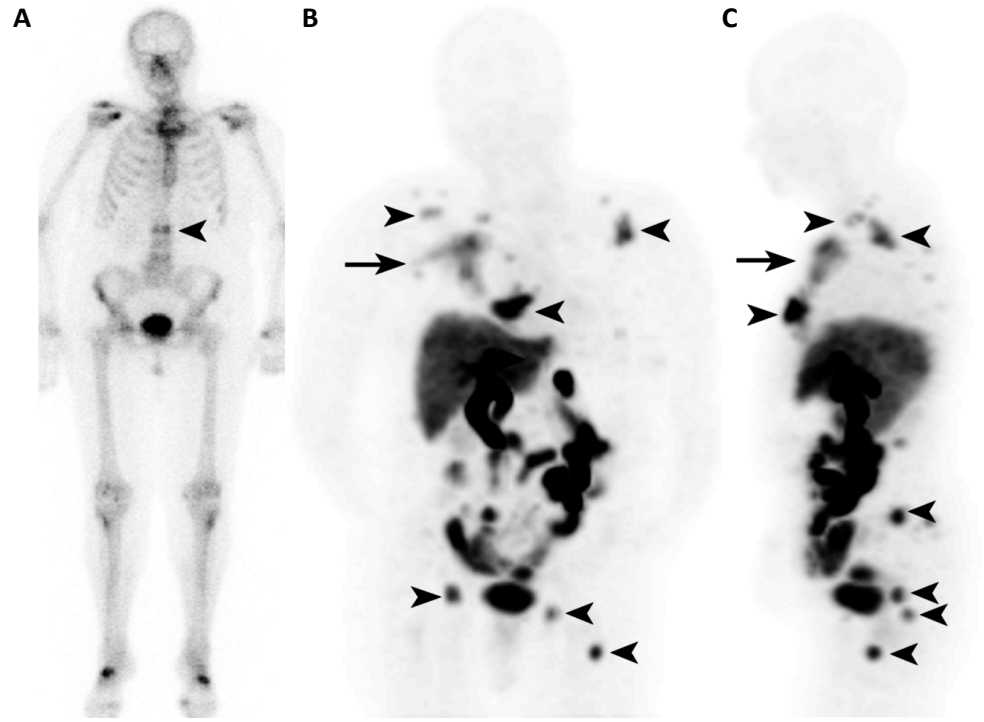

Figure 2. Bone scan (A) of patient showing suggestive lesion at L2 (arrowhead). Biopsy of this lesion did not show malignancy. Coronal (B) and sagittal (C) images of ^{18}F-FES-PET showing ^{18}F-FES-uptake in vertebra L2 and multiple other bone metastases (arrowheads), as well as large locoregional recurrence in soft tissue (arrow). Only most intense lesions are indicated.

^{18}F-FES-PET to evaluate ER expression after progression on antihormonal therapy

Ten patients underwent ^{18}F-FES-PET to evaluate their ER status after progression on antihormonal therapy. In these patients there was no consensus whether to give chemotherapy or antihormonal therapy. ^{18}F-FES-PET showed increased ^{18}F-FES uptake in one or more metastatic lesions in all 10 patients. In six of these there was partial discordance with absence of ^{18}F-FES uptake in some metastases. In four patients, intended chemotherapy was switched to antihormonal therapy based on high ^{18}F-FES uptake. One patient with signs of bone marrow invasion on ^{18}F-FES-PET and accompanying thrombocytopenia was switched to chemotherapy (figure 3).

^{18}F-FES-PET to differentiate between metastases originating from different tumor types

In two patients definite malignant lesions were detected by conventional imaging. It was uncertain whether these originated from the earlier ER positive breast tumor, or from another tumor type. One patient presented with a radicular syndrome in the region of root C6, 5 years after primary breast cancer diagnosis. Neurological examination and MRI

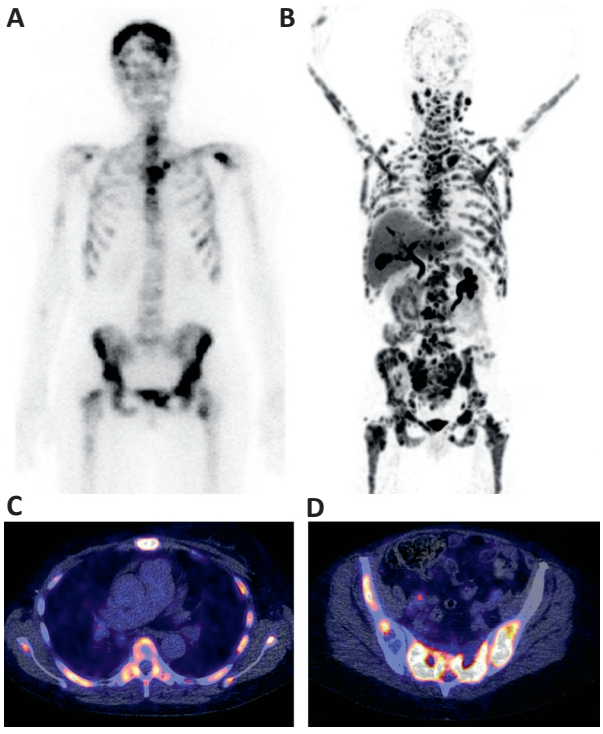

Figure 3. Bone scan (A), ^{18}F-FES-PET (B), and ^{18}F-FES-PET/CT (C and D) images of patient with progressive disease after multiple lines of antihormonal therapy and chemotherapy. Extensive tracer uptake was seen in bone, lymph nodes, and skull. Interestingly, ^{18}F-FES-uptake seemed to be predominant in bone marrow of this patient, in whom laboratory signs of bone marrow infiltration were present.

indicated pathologic compression at vertebra C6, suspected to be due to a metastasis or plasmacytoma. A biopsy to prove the origin of the metastasis was considered too risky. ^{18}F-FES-PET showed uptake in C6 and Th4 (figure 4). No other tumor lesions were observed. Therefore this patient received antihormonal therapy for metastatic disease and radiotherapy. A second patient, known with an ER negative second tumor, presented with a single metastasis in the humerus. Too little material was obtained by biopsy to differentiate between the ER positive and ER negative primary. ^{18}F-FES-PET showed no lesional uptake, suggesting an origin from the ER negative tumor. Therefore this patient did not receive antihormonal therapy.

Questionnaires

All 3 questionnaires in the 33 patients were fully completed. Referring physicians reported an improved diagnostic understanding in 29 of the 33 patients (88%). In 18 of these, this was the consequence of ^{18}F-FES-PET solely, while in 11 patients other factors were equally or more important. Diagnostic understanding was independent of the indication for ^{18}F-FES-PET

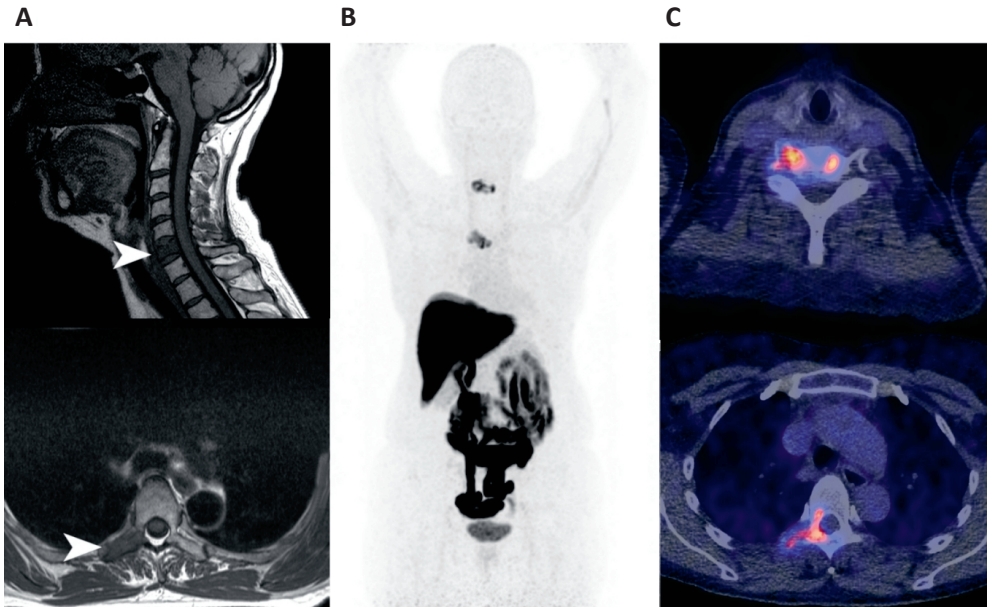

Figure 4. This patient presented with neurologic symptoms of root C6 5 y after primary breast cancer diagnosis. On MRI (A), pathologic processes were suspected in C6 and T4. Biopsy to prove that these were metastases and originated from prior primary breast cancer was considered too risky. ^{18}F-FES uptake was observed in suspected metastases in C6 and T4 (B). ^{18}F-FES-PET/CT images (C) matched MRI findings. No other pathologic uptake was observed.

(*P*=0.12), but was superior for positive compared to negative ^{18}F-FES-PET results (*P*=0.002).

A change in therapeutic strategy based on ^{18}F-FES-PET was reported in 16 (48%) of the 33 patients. In 11 of them, information provided by ^{18}F-FES-PET was very important in leading to a change in therapy, while in five patients other factors were equally or more important. Showing ^{18}F-FES uptake in equivocal lesions led to the initiation of radiotherapy (n=4), bisphosphonates (n=1), and antihormonal therapy (n=3). Presence of ^{18}F-FES uptake in known metastases led to the initiation of a new line of antihormonal therapy (n=4). Absence of ^{18}F-FES uptake in known metastases and in equivocal lesions respectively led to refraining from antihormonal therapy (*n* =2), and refraining from radiotherapy (n=2) (table 3).

DISCUSSION

This is the first study evaluating the value of ^{18}F-FES-PET in breast cancer patients presenting with a clinical dilemma unresolved after conventional work-up. It shows that whole-body imaging of ER expression with ^{18}F-FES-PET can be a valuable additional diagnostic tool when conventional work-up is ambiguous and biopsies are not feasible or inconclusive.

Table 3: Changes in therapeutic strategy following ^{18}F-FES-PET

Intended therapy	^{18}F-FES-PET finding	Final therapy	***Patients (n)***
Chemotherapy	Strong ER positivity	Endocrine therapy	4
Wait and see policy	PET positive relapse	Endocrine therapy	3
Endocrine therapy	New bone metastases	+ Radiotherapy	4
Endocrine therapy	New bone metastases	+ Bisphophonates	1
Endocrine therapy	Negative ^{18}F-FES-PET	Chemotherapy	2
Radiotherapy	Negative ^{18}F-FES-PET	Wait and see policy	2

Based on the questionnaires, the referring physicians reported an improved diagnostic understanding in 88% of all 33 questionnaires, which supported therapy changes in 48%. We retrospectively divided patients that underwent ^{18}F-FES-PET in three groups. The first group underwent ^{18}F-FES-PET to establish a diagnosis in case of equivocal or conflicting conventional work-up. Recent guidelines (ESMO/ NCCN) suggest that ^{18}F-FDG-PET can be considered in case of equivocal imaging, although biopsy of suspicious sites is more likely to provide useful information.[105,106] In our patients, ^{18}F-FDG-PET was rarely requested for various reasons, e.g. positive ^{18}F-FDG-PET would not exclude inflammatory disease, or would not differentiate between metastases from ER positive breast cancer or another cancer origin.[111,112] ^{18}F-FES-PET has been shown to detect ER positive metastases with high specificity.[10,16,96] Therefore this technique may be used as surrogate for tissue biopsy in case lesions are difficult to access. In our study in a number of patients biopsies were not feasible or inconclusive. We showed that ^{18}F-FES-PET could be used to prove the presence of ER positive metastases in case of equivocal conventional work-up. ^{18}F-FES-PET can, however, not be used to exclude metastases in general as ER negative metastases may be present.

In light of a possible conversion in ER phenotype, know-how of ER expression can potentially facilitate the choice between chemotherapy and antihormonal therapy. Single biopsy studies have shown conversion from an ER positive to ER negative phenotype in up to 30% of the patients. In addition, ^{18}F-FES-PET studies have shown ^{18}F-FES-negative disease in 32-53% of the patients, which was highly predictive of failure of response to antihormonal therapy. We evaluated ER expression in 10 patients in which a biopsy was problematic. All 10 had presence of ^{18}F-FES-positive metastases. Interestingly, our results indicated that an extensive variance in ^{18}F-FES uptake can be present across positive lesions within individuals (coefficient of variance 68% ± 4%). This heterogeneity matches with findings of multiple biopsies from individual patients showing a variety of ER levels can be present.[113,114] Furthermore, 45% of the patients with a positive ^{18}F-FES-PET had both ^{18}F-FES-positive as well as ^{18}F-FES-negative metastases. This was higher than previously reported (10-24%).[70] This points towards the relevance of ^{18}F-FES-PET, as it provides knowledge of whole-body tumor ER expression. So far, the consequence of a heterogeneous ER expression for therapy-management has received strikingly little attention in the clinic and deserves further exploration.

The last group of patients in our study underwent ^{18}F-FES-PET to differentiate between distant recurrences originating from the earlier ER positive primary breast tumor and metastases originating from a second tumor. The ER specific ^{18}F-FES tracer permitted non-invasive differentiation between tumor types avoiding the necessity of additional (invasive) diagnostic procedures and leading to early institution of the right drug.

Our study and approach has limitations. We provide an analysis of the value of ^{18}F-FES-PET in the standard clinical situation. The standardized questionnaires we used can still be subject to bias and should be interpreted with caution. Therapy changes were carefully made taking into account earlier therapies, other imaging results and clinical presentation. Imaging analysis was performed retrospectively which can lead to potential bias. To minimize bias favoring ^{18}F-FES-PET we performed a central revision of all conventional imaging. Due to the absence of a clear golden standard, sensitivities and specificities cannot be given. Prospective studies should be performed to prove that ^{18}F-FES-PET can replace the biopsy for treatment decisions.

Current ^{18}F-FES-PET studies do not describe its capacity to detect liver metastases. The physiological uptake in the liver due to metabolization well exceeds the ^{18}F-FES uptake that is seen in the uterus or most ER positive metastases.[65] In our study, the detection of liver metastases by ^{18}F-FES-PET was poor, and one histological ER positive metastasis was not detected. We did observe focal cold lesions in two other patients. Quantification of ^{18}F-FES-uptake in these lesions was hampered by the high physiological uptake in surrounding tissue. As liver biopsies were not available in these patients, it is unknown whether the cold appearance can be explained by focal loss of ER expression. ^{18}F-FES-negative liver metastases should therefore be evaluated by immunohistochemistry in future studies.

There are a number of factors other than ER expression that might affect ^{18}F-FES uptake. Small metastases may not show ^{18}F-FES uptake due to resolution limitations of PET. In addition, presence of estrogen analogues such as tamoxifen can block tumor ^{18}F-FES uptake.[67] For this reason we choose an arbitrary drug withdrawal period of 5 weeks for ER ligands. Patients that discontinued fulvestrant 5 weeks prior to ^{18}F-FES-PET had a high rate of ^{18}F-FES-negative lesions (14 out of 20 metastases). A 5-week drug withdrawal period may therefore not have been sufficient to exclude occupancy of ERs by this drug with a long half-life of 40 days. The only premenopausal patient in this study had ^{18}F-FES uptake values well below the 95% confidence interval of postmenopausal patients. In a previous study in primary breast cancer patients only 6 out of 10 patients with ER positive tumors showed focal ^{18}F-FES uptake.[71] Although not mentioned in the discussion of this report, it is of interest that all four patients with a false-negative ^{18}F-FES-PET were most likely premenopausal as their age ranged from 34-45 years, while the age of the six patients with a true-positive ^{18}F-FES-PET ranged from

56-71 years old. Together this data underlines the possibility of background estrogens levels to influence ^{18}F-FES uptake, which warrants further exploration.

CONCLUSION

Whole body imaging of ER expression with ^{18}F-FES-PET can aid diagnosis and support treatment decision-making in ER positive breast cancer patients presenting with a variety of diagnostic dilemmas. Based on our results, we do not recommend ^{18}F-FES-PET to evaluate liver metastases. The therapeutic consequences of having heterogeneous ^{18}F-FES uptake and the influence of background estrogen levels should further be explored.

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